Intestinal and Hepatobiliary Transport of Ximelagatran and Its Metabolites in Pigs

Elin Sjödin, Holger Fritsch,1 Ulf G. Eriksson, Ulrika Logren, Anders Nordgren, Patrik Forsell, Lars Knutson, and Hans Lennernäs

Department of Pharmacy, Uppsala University, Uppsala, Sweden (E.S., H.L.); Department of Surgical Sciences, University Hospital, Uppsala, Sweden (A.N., P.F., L.K.); and AstraZeneca R&D Mölndal, Mölndal, Sweden (H.F., U.E., U.L.)

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ABSTRACT:
The direct thrombin inhibitor melagatran is formed from ximelagatran via two intermediate metabolites, OH-melagatran and ethylmelagatran. The biotransformation of ximelagatran does not involve cytochrome P450 isoenzymes, and it has been suggested that a reported interaction with erythromycin may instead be mediated by transport proteins. A pig model that simultaneously enables bile collection, sampling from three blood vessels and perfusion of a jejunal segment, was used to investigate the biotransformation of ximelagatran and the effect of erythromycin on the intestinal and hepatobiliary transport of ximelagatran and its metabolites. The pigs received enteral ximelagatran (n = 6), enteral ximelagatran together with erythromycin (n = 6), i.v. ximelagatran (n = 4), or i.v. melagatran (n = 4). The plasma exposure of the intermediates was found to depend on the route of ximelagatran administration. Erythromycin increased the area under the plasma concentration-time curve (AUC) of melagatran by 45% and reduced its biliary clearance from 3.0 ± 1.3 to 1.5 ± 1.1 ml/min/kg. Extensive biliary exposure of melagatran and ethylmelagatran, mediated by active transport, was evident from the 100- and 1000-fold greater AUC, respectively, in bile than in plasma. Intestinal efflux transporters seemed to be of minor importance for the disposition of ximelagatran and its metabolites considering the high estimated fobs of ximelagatran (80 ± 20%) and the negligible amount of the compounds excreted in the perfused intestinal segment. These findings suggest that transporters located at the sinusoidal and/or canicular membranes of hepatocytes determine the hepatic disposition of ximelagatran and its metabolites, and are likely to mediate the ximelagatran-erythromycin pharmacokinetic interaction.

Ximelagatran was the first oral direct thrombin inhibitor to reach the market. As an oral anticoagulant, it had the potential to become an alternative to warfarin and other vitamin K antagonists; however, because of safety concerns involving hepatotoxicity, ximelagatran was withdrawn in 2006. Warfarin currently has an important role in the prevention and treatment of thromboembolic events but is unfortunately also associated with a variable pharmacokinetic and pharmacodynamic response, delayed onset and offset of action, numerous drug-drug interactions, and serious safety issues (Hawkins, 2004; Greenblatt and von Molke, 2005). The narrow therapeutic index of warfarin and the many sources of variability require monitoring of the hemorrhagic and thrombotic functions of melagatran were protected by introducing an ethyl ester residue and the less basic N-hydroxylated amidine (Fig. 1), a prodrug strategy that increased the bioavailability from 5.8 ± 2.3 to 21.9 ± 1.7% and reduced the interindividual variability in humans (Eriksson et al., 2003). The improved oral bioavailability was explained by the greater intestinal permeability to ximelagatran than to melagatran, reflected by the 80-fold increase in Caco-2 cell permeability and the 160-fold higher octanol-water partition coefficient (at pH 7 for ximelagatran and pH 9.7 for melagatran) (Gustafsson et al., 2001).

The biotransformation of ximelagatran to melagatran requires two metabolic processes: hydrolysis of the ethyl group on the carboxylic acid and reduction of the hydroxyl group on the amidine (Fig. 1) (Clement and Lopian, 2003; Eriksson et al., 2003). Carboxylesterases (CESs) mediate the hydrolysis, whereas the enzyme system catalyzing the N-hydroxylamidine residue consists of three components: cytochrome b5, its reductase, and a third unidentified protein (Clement and Lopian, 2003; Andersson et al., 2005). Two intermediate metabolites, OH-melagatran and ethylmelagatran, occur during the formation of melagatran. In vitro and in vivo investigations have shown that neither ximelagatran nor its metabolites are substrates or inhibitors of any major cytochrome P450 isoenzymes (Bredberg et al., 2003). However, despite this, erythromycin increased the plasma concentrations of melagatran by promoting the hydrolysis of the ethyl ester residue and reducing the interindividual variability in humans (Eriksson et al., 2003). The improved oral bioavailability was explained by the greater intestinal permeability to ximelagatran than to melagatran, reflected by the 80-fold increase in Caco-2 cell permeability and the 160-fold higher octanol-water partition coefficient (at pH 7 for ximelagatran and pH 9.7 for melagatran) (Gustafsson et al., 2001).

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1 Current affiliation: Boehringer Ingelheim GmbH, Ingelheim, Germany.

ABREVIATIONS: CES, carboxylesterase; VP, portal vein; VH, hepatic vein; VF, femoral vein; P-gp, P-glycoprotein; MRP, multidrug resistance associated protein; OATP, organic anion transporting protein; P, part; T, treatment; LC, liquid chromatography; MS, mass spectrometry; LLOQ, lower limit of quantification; AUC, area under the plasma/bile concentration-time curve.
of OH-melagatran, ethylmelagatran, and melagatran following concomitant administration with ximelagatran in healthy subjects (Eriksson et al., 2006). Data from cell culture and rat biliary excretion models suggested that the interaction was caused by erythromycin inhibiting the biliary excretion of melagatran (Eriksson et al., 2006).

In this study, a pig model that simultaneously enables bile collection, intestinal perfusion, and plasma sampling from the portal vein (VP), hepatic vein (VH), and femoral vein (VF) was used to better understand the pharmacokinetics of ximelagatran and its metabolites, and the interaction with erythromycin. Pigs were chosen as the animal model for several reasons: first, the anatomical and physiological parameters of the porcine gastrointestinal tract resemble those in humans (Krishtan et al., 1994; Kararli, 1995; Nejdfors et al., 2000). Second, the expression and activity of the major enzymes responsible for drug biotransformation are comparable with those in humans, thus making the pig a useful model for metabolic studies (Anzenbacher et al., 1998; Zuber et al., 2002). Less is known about the expression of transport proteins in pigs, although the pharmacokinetic properties of drugs known to be actively transported in humans (e.g., cyclosporine, digoxin, fexofenadine, verapamil, danazol) are similar (Petri et al., 2006; Tannergren et al., 2006; Persson et al., 2008). The possible use of porcine proximal tubular cells and brain capillary endothelial cells for mimicking drug transport in the human kidney and blood-brain barrier, respectively, is under investigation (Eisenblätter and Galla, 2006; Tannergren et al., 2006; Persson et al., 2008). The possible use of porcine proximal tubular cells and brain capillary endothelial cells for mimicking drug transport in the human kidney and blood-brain barrier, respectively, is under investigation (Eisenblätter and Galla, 2006; Tannergren et al., 2006; Persson et al., 2008). The possible use of porcine proximal tubular cells and brain capillary endothelial cells for mimicking drug transport in the human kidney and blood-brain barrier, respectively, is under investigation (Eisenblätter and Galla, 2006; Tannergren et al., 2006; Persson et al., 2008).

Materials and Methods

Animals. The study protocol was approved by the local ethics committee for animal experiments, and the handling of the animals followed national regulations. The average weight of the 20 animals, which were 10 to 12 weeks old and of mixed breed (Hampshire, Yorkshire, and Swedish Landrace), was 27.7 ± 2.90 kg (mean ± S.D.). Food was withheld over the night before the experiment, but water was allowed ad libitum.

Study Design and Investigational Drugs. The study consisted of two parts: in part one (PI), the pharmacokinetic interaction between single-dose ximelagatran and erythromycin was investigated, and in part two (PII), the pharmacokinetics of ximelagatran and melagatran were studied following separate i.v. single-bolus doses (Table 1).

In PI, 10 male and 2 female pigs received 200 mg of ximelagatran [treatment I (TI); n = 6] or 250 mg of erythromycin 20 min before 200 mg of ximelagatran (TII; n = 6). The doses of the investigational drugs were selected based on results from a previous pilot study (n = 2) (data not presented here). The drugs were given in solution at room temperature as single-bolus doses into the proximal jejunum via a Loc-I-Gut perfusion tube (Synectics Medical, Stockholm, Sweden). This tube has been designed to allow simultaneous administration of drugs, intestinal perfusion, and sampling of biliary fluid in humans and large mammals (Knutson et al., 1989; Lennerna et al., 1992; Petri et al., 1992).

Ximelagatran (AstraZeneca R&D, Möln达尔, Sweden) was dissolved in acidified isotonic sodium chloride (at pH 4 for stability reasons) to a final concentration of 4.44 mg/ml (45-ml dose). Erythromycin was prepared from erythromycin lactobionate (Abbotcin; Abbot Scandinavia AB, Solna, Sweden), which was dissolved in isotonic sodium chloride in accordance with instructions from the manufacturer to a final erythromycin concentration of 5 mg/ml (50-ml dose).

Following each drug administration, the syringe and the Loc-I-Gut catheter were rinsed with 50 ml of isotonic sodium chloride (37°C) to ensure that the complete dose was administered. Possible leakage of the enteral dose into the perfused intestinal segment was monitored by adding 18.5 kBq of the nonabsorbable marker [14C]polyethylene glycol 4000 (Amersham Biosciences, Buckinghamshire, UK) to the drug solutions.

In PII, male pigs received single-bolus i.v. doses of either 15 mg of ximelagatran (TIII; n = 4) or 15 mg of melagatran (TIV; n = 4) in a peripheral vein in the ear. The ximelagatran i.v. solution was prepared as described above, but to a final concentration of 5 mg/ml, and was then sterile-filtered. Melagatran was obtained in prefilled syringes containing 10 mg/ml melagatran solution for injection (Melagatran AstraZeneca; AstraZeneca AB, Södertälje, Sweden).

The single-pass perfusion of a 10-cm-long intestinal segment applied for collection of intestinal secretion was started at the same time that the study drug was given. In both PI and PII, a prewarmed (37°C) phosphate buffer at pH 6.5 and 290 mM NaCl, 10 mM NaH₂PO₄, 63 mM Na₂HPO₄, 34 mM NaCl, 1.0 g/l polyethylene glycol 4000, and 35 mM mannitol. At a flow rate of 2 ml/min using a syringe pump (model 355; Sage Instruments, Cambridge, MA), the phosphate buffer (5.4 mM KCl, 48 mM NaCl, 10 mM NaH₂PO₄, 63 mM Na₂HPO₄, 34 mM NaCl, 1.0 g/l polyethylene glycol 4000, and 35 mM mannitol) and flow rate have previously been used and validated in several in vivo perfusion studies (e.g., Lennerna et al., 1992; Petri et al., 2006). This study, 0.2 mg/mg phenol red (Sigma-Aldrich, Stockholm, Sweden) was used as a nonabsorbable volume marker (Sutton et al., 2001).
mg/kg atropine (Atropin NM Pharma, 0.5 mg/ml; Merck NM AB, Stockholm, Sweden), and a mixture of 3 mg/kg ketamine and 3 mg/kg zolazepam (Zoletil; Virbac S.A., Carros, France). Morphine, 1 mg/kg (Morfim Meda, 10 mg/ml; Meda AB, Solna, Sweden), was also administered i.v. into a peripheral vein. The pigs underwent tracheotomy and were mechanically ventilated with an oxygen-air mix by means of a Servo 900C ventilator (Siemens-Elema, Solna, Sweden). The tidal volume was adjusted to keep the arterial partial pressure of carbon dioxide between 5.0 and 6.0 kPa. During the experiment, the animals were kept sedated and anesthetized by continuous i.v. administration of 0.5 mg/kg/h morphine, 0.25 mg/kg/h pancuronium bromide (Pavulon, 2 mg/ml; Organon AB, Gothenburg, Sweden), and 20 mg/kg/h ketamine (Ketaminol Vet, 100 mg/ml; Intervet, Stockholm, Sweden). To provide rehydration and maintain fluid balance, Rehydrex with 25 mg/ml glucose (Fresenius Kabi AB, Stockholm, Sweden), and a mixture of 3 mg/kg tiletamine and 3 mg/kg zolazepam (Zoletil; Virbac S.A., Carros, France). Morphine, 1 mg/kg (Morfim Meda, 10 mg/ml; Meda AB, Solna, Sweden), was also administered i.v. to replace the fluid lost from blood sampling. If the central venous pressure decreased, additional Ringer’s acetate was administered i.v. The body temperature of the animals was maintained by using a thermostat-controlled heating pad. Blood gases, electrocardiograms, heart rate, and arterial and central venous pressures were monitored throughout the experiment to ensure normal physiological values. By the end of the study, while still under anesthesia, the animals were given a lethal i.v. dose of 20 to 30 mmol of potassium chloride.

Surgical Procedures. A catheter was introduced via the left external jugular vein to the right VH under fluoroscopic control, and its position was confirmed by contrast injection. The abdominal cavity was opened through a midline incision, and a catheter was placed in the common hepatic duct proximal to the gall bladder. Distal to the insertion of the catheter, a ligature was placed around the bile duct to prevent bile from entering the intestine. The superior mesenteric vein was cannulated, and a catheter was advanced to the VP. A catheter was also introduced in the right VF. Urine and gastric fluid were removed by catheters inserted in the urinary bladder and stomach, respectively. An incision was made in the duodenum to enable insertion of the Loc-I-Gut perfusion tube (Synetics Medical, Stockholm, Sweden), which was positioned in the proximal jejunum (Knutson et al., 1989; Lennernas et al., 1992; Petri et al., 2006). Once in place, the two balloons attached to the Loc-I-Gut tube were inflated to create a 10-cm-long intestinal segment. The animals were stabilized for about 30 min after surgery before the study drugs were administered distal to the perfused segment. During the stabilization period, the enteral segment was rinsed with prewarmed (37°C) isotonic sodium chloride. The surgical procedures lasted about 90 min and were performed by experienced personnel at a validated laboratory.

Collection of Biological Samples. The collection of biological samples is summarized in Table 1. Venous blood samples were drawn from three blood vessels: the VP, VH, and VF. The samples were taken predose and 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 240, 300, and 360 min after enteral or i.v. administration of ximelagatran or melagatran. Plastic 2.7-ml tubes containing buffered sodium citrate solution (BD Vacutainer Citrate Tubes; Becton Dickinson AB, Stockholm, Sweden) were used for collection of blood samples intended for determination of ximelagatran, OH-melagatran, ethylmelagatran, and melagatran concentrations. The blood and citrate were carefully mixed before centrifugation of the samples at 4°C for 20 min at a centrifugal force of 1500g (Universal 16R; Hettich, Tuttingen, Germany). The obtained plasma was transferred to 3-ml cryovials (Simport Cryovials; Simport Plastics Ltd., Belloeil, QC, Canada), immediately frozen, and stored at −20°C until analyzed. The biliary fluid was continuously collected from the cannulated bile duct at 20-min intervals throughout the experiment. The bile was weighed and transferred to 3-ml cryovials (Simport Cryovials, Simport Plastics Ltd.). To increase the stability of the analytes, 1 M HCl was added (80 μl of HCl to 1 ml of bile) before storage at −70°C. The perfusate leaving the intestinal segment was continuously collected on ice at 10- or 20-min intervals, depending on the length of the perfusion. In the VP, the enteral perfusate was finalized 100 min after administration of ximelagatran, and in PI the perfusate lasted throughout the experiment, i.e., 360 min following i.v. dosing of ximelagatran or melagatran. The perfusate samples were weighed, transferred to 3-ml cryovials (Simport Cryovials, Simport Plastics Ltd.), and frozen at −20°C pending analysis. Liver samples were taken directly from the PI pigs (TI and TII) after the i.v. potassium chloride overdose, quickly frozen in liquid nitrogen, and stored at −70°C.

Bioanalysis. The concentrations of ximelagatran, OH-melagatran, ethylmelagatran, and melagatran in plasma, bile, perfusate, and liver samples were determined at Development Drug Metabolism and Pharmacokinetics and Bioanalysis (AstraZeneca R&D Möln达尔). The method used to determine plasma concentrations, which has been previously published, comprised mixed-mode solid-phase extraction for sample preparation and determination by liquid chromatography (LC), followed by positive electrospray ionization mass spectrometry (MS) (Dunér et al., 2007). The lower limit of quantification (LLOQ) for all four analytes was 0.0100 μM plasma. The calibration range was 0.0100 to 4.0 μM plasma, and the imprecision, given as the relative S.D., was less than 20% at 0.0100 μM and less than 5% at 4.0 μM for all four analytes during the study.

The acidified bile samples and the perfusate samples were centrifuged and diluted with a mixture of 10% acetonitrile and 90% buffer (10 mM ammonium acetate and 5 mM formic acid). The concentrations of ximelagatran, OH-melagatran, ethylmelagatran, and melagatran were determined by LC/MS using the same internal standards, pipetting robot, and LC/MS equipment as those described for plasma (Dunér et al., 2007). There were, however, some differences from the plasma methodology. The samples were diluted directly, and the internal standards were added without previous solid-phase extraction sample preparation. The LC gradient was also slightly modified; the analytes were eluted by a linear gradient, ranging from 10 to 34.5% acetonitrile, followed by 80% acetonitrile to wash the column between each injection. The LLOQ for all four analytes was 0.100 μM bile/perfusate. The calibration range was 0.100 to 98 μM bile/perfusate, and the imprecision, given as the relative S.D., was less than 20% at 0.100 μM and less than 5% at 98 μM for all four analytes during the study.

The liver samples were prepared by adding two parts of Krebs’ buffer (2 g/l Na-glucose, 0.141 g/l anhydrous MgSO4, 0.16 g/l KH2PO4, 0.35 g/l KCl, 6.9 g/l NaCl, 0.373 g/l CaCl2, and 2.1 g/l NaHCO3) before homogenization of the samples. The homogenates were mixed with acidified acetonitrile (3 ml/g liver; 1% of 25% HCl and 99% acetonitrile), centrifuged, and stored at −70°C pending analysis. The diluted liver homogenates were mixed with internal standard solution before analyzing the concentrations of ximelagatran, OH-melagatran, ethylmelagatran, and melagatran with LC/MS as described in a method for human urine samples (Larsson et al., 2003). The LLOQ for all four analytes was 0.0100 μM diluted liver homogenate. The calibration range was 0.0100 to 4.00 μM acidified homogenate, and the imprecision, given as the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Animals</th>
<th>Study Drugs</th>
<th>Route of Administration</th>
<th>Doses</th>
<th>Sampling Site</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I: TI</td>
<td>6</td>
<td>Ximelagatran</td>
<td>Jejunum</td>
<td>200</td>
<td>Plasma (VP, VH, VF)</td>
<td></td>
</tr>
<tr>
<td>Part I: TII</td>
<td>6</td>
<td>Ximelagatran + erythromycin</td>
<td>Jejunum</td>
<td>200 - 250</td>
<td>Bile</td>
<td></td>
</tr>
<tr>
<td>Part II: TIII</td>
<td>4</td>
<td>Ximelagatran</td>
<td>i.v.</td>
<td>15</td>
<td>Intestinal Perfusion</td>
<td></td>
</tr>
<tr>
<td>Part II: TIV</td>
<td>4</td>
<td>Melagatran</td>
<td>i.v.</td>
<td>15</td>
<td>Liver Samples</td>
<td></td>
</tr>
</tbody>
</table>
relative S.D., was less than 20% at 0.0100 μM and less than 5% at 4.00 μM for all four analytes.

Data Analysis. Pharmacokinetic variables were calculated from the plasma (VP, VH, and VF) and bile concentration-time profiles of ximelagatran, OH-melagatran, ethylmelagatran, and melagatran. The following pharmacokinetic variables were determined with noncompartmental analysis using WinNonlin 4.0 (Pharsight Corp., Mountain View, CA): the area under the plasma/bile concentration versus time curve (AUC0–t, e,bile), the area under the moment plasma concentration versus time curve (AUMC0–t), the maximum plasma/bile concentration (Cmax), the time to reach Cmax (Tmax), the terminal rate constant, and the elimination half-life (t1/2). The AUC was calculated by means of the linear/logarithmic trapezoidal rule, and the terminal rate constant was determined by log-linear regression analysis of the last three to five concentration–time points. The AUC beyond the last time point (t = 6 h) was obtained by dividing the last predicted concentration by the terminal rate constant. The apparent hepatic extraction ratio (Ehi) (eq. 1) was calculated for ximelagatran based on the differences in AUC between the VP and VH. The apparent hepatic clearance (CLhi) (eq. 2) was calculated from the Ehi.

\[
E_{hi} = \frac{AUC_{0–t, VP} - AUC_{0–t, VH}}{AUC_{0–t, VP}}
\]

The hepatic blood flow (Qhi, 52 ml/min/kg) had been previously calculated using the constant dye infusion technique and the same anesthetic regimen on pigs of the same breed and age (Nordgren et al., 2002). The blood-to-plasma ratio of ximelagatran (0.66) was included to convert the blood clearance into plasma clearance (data on file, AstraZeneca R&D). The total molar amount excreted in bile (A e,bile) during the 6-h experiment was calculated for each of the four compounds:

\[
A_{e,bile} = \sum (C_{e,bile} \times V_{e,bile})
\]

where C e,bile is the concentration of each compound in bile, and V e,bile is the volume of the biliary fluid in every 20-min fraction. The amount excreted in bile, expressed as the fraction of the administered dose, was calculated for ximelagatran, the intermediates, and melagatran separately and for the sum of the four compounds. Following i.v. administration of ximelagatran and melagatran, the amount excreted in bile can be referred to as the fraction of the dose eliminated in bile (f e,bile) because the entire dose given was systemically available, the sampling time was sufficiently long in relation to the half-lives, and no reabsorption from the gut lumen of the poorly permeable ethylmelagatran and melagatran was expected:

\[
f_{e,bile} = \frac{A_{e,bile}}{dose}
\]

The A e,bile of ximelagatran and its metabolites expressed in molar amounts was used to estimate the apparent biliary clearance (CL e,bile) (eq. 5) and the fraction of dose absorbed (fabs) (eq. 6).

\[
CL_{e,bile} = \frac{A_{e,bile}}{AUC_{0–t, VP}}
\]

\[
f_{abs} = \frac{\sum A_{e,bile,enteral}}{\sum A_{e,bile,i.v.}} \times \frac{dose_{i.v.}}{dose_{enteral}}
\]

The apparent CL e,bile was calculated for each of the four compounds separately, and the f abs was estimated from the summed amount of ximelagatran, intermediates, and melagatran excreted in bile. This way of using cumulative bile data to estimate f abs is based on the assumption that the fraction of the sum of ximelagatran and its three metabolites eliminated via bile remains constant, irrespective of route of administration. The i.v. administration of ximelagatran and melagatran also enabled determination of their bioavailability (F) in pigs (eq. 7). The bioavailability of melagatran was calculated from the plasma AUC0–6 of melagatran following enteral administration of ximelagatran and i.v. administration of melagatran.
after Enteral Administration of Ximelagatran with and without that intestinal metabolism occurred after absorption across the apical metabolism. The high degree of absorption of ximelagatran suggests differences between the group of pigs receiving ximelagatran (TI) and that administration was accompanied by a shorter formation of ximelagatran differed between the intestine and the liver. suggests that the activity of the enzymes involved in the biotransformation of Ximelagatran after Enteral and i.v. Administration was accompanied by a shorter absorption across the apical metabolism. The high degree of absorption of ximelagatran suggests

\[
C_{\text{max}} (\mu M) = \begin{bmatrix}
3.12 & 1.43 & 2.11 & 1.33 \\
1.63 & 0.68 & 1.09 & 0.47 \\
1.23 & 0.53 & 0.86 & 0.37 \\
0.20 & 0.12 & 0.30 & 0.16 \\
0.20 & 0.12 & 0.30 & 0.16 \\
0.20 & 0.12 & 0.30 & 0.16 \\
\end{bmatrix}
\]

\[\begin{align*}
\text{TI} & : 7.34 \pm 2.22 & 4.17 \pm 2.55 & 3.67 \pm 0.72 \\
\text{VH} & : 5.09 \pm 2.03 & 4.83 \pm 1.28 & 0.37 \pm 0.16 & 0.99 \pm 1.28 \\
\text{VF} & : 4.27 \pm 1.55 & 3.76 \pm 0.72 & 0.51 \pm 0.15 & 0.86 \pm 0.95 \\
\end{align*}\]

\[\begin{align*}
\text{TI} & : 7.15 \pm 1.23 & 10.3 \pm 2.86 \\
\text{VH} & : 6.88 \pm 1.06 & 9.78 \pm 2.68 \\
\text{VF} & : 6.30 \pm 1.03 & 9.17 \pm 2.52 \\
\end{align*}\]

\[\begin{align*}
\text{TI} & : 3.93 \pm 1.90 & 0.33 \pm 0.18 & 0.41 \pm 0.39 \\
\text{VH} & : 2.64 \pm 0.86 & 0.26 \pm 0.16 & 0.46 \pm 0.54 \\
\text{VF} & : 2.02 \pm 0.70 & 0.26 \pm 0.13 & 0.37 \pm 0.36 \\
\end{align*}\]

\[\begin{align*}
\text{TI} & : 1.33 \pm 0.22 & 1.0 \pm 0.20 & 1.9 \pm 0.20 \\
\text{VH} & : 1.0 \pm 0.20 & 1.0 \pm 0.20 & 2.5 \pm 1.2 & 1.9 \pm 0.3 \\
\text{VF} & : 1.0 \pm 0.20 & 1.0 \pm 0.20 & 2.5 \pm 1.2 & 1.9 \pm 0.3 \\
\end{align*}\]

\[p < 0.05.\]

**Results**

**Biotransformation of Ximelagatran after Enteral and i.v. Administration.** Ximelagatran was metabolized to melagatran via two intermediate metabolites: OH-melagatran and ethylmelagatran (Fig. 1). The plasma exposure of the intermediates was dependent on whether ximelagatran was introduced enterally or i.v. (Fig. 2). The plasma AUC ratios for OH-melagatran/melagatran and ethylmelagatran/melagatran were 69 and 8% after enteral administration of ximelagatran and 5 and 55% after i.v. administration (Tables 2 and 5). The reduction in the plasma AUC of OH-melagatran following i.v. administration was accompanied by a shorter \(t_{1/2} \) (0.7 \pm 0.3 h after i.v. and 1.4 \pm 0.4 h after enteral administration; \(p < 0.05\)). These results suggest that the activity of the enzymes involved in the biotransformation of ximelagatran differed between the intestine and the liver.

**Part I: Pharmacokinetics of Ximelagatran and Its Metabolites after Enteral Administration of Ximelagatran with and without Erythromycin.** Erythromycin increased the absorption of melagatran by approximately 45% in the VP, VH, and VF (\(p < 0.05\)), and a similar increase was observed in the \(C_{\text{max}}\) of melagatran (Table 2). In the peripheral vein, the bioavailability of melagatran was increased from 18 ± 3.2% to 24 ± 6.9% by erythromycin (\(p < 0.05\)), and this increase was also seen in the VP and VH (Table 3). No relevant changes in the \(T_{\text{max}}\) or the elimination \(t_{1/2}\) of ximelagatran and its metabolites were observed, suggesting that the interaction between erythromycin and melagatran occurred during the first pass (Table 2).

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Ximelagatran</th>
<th>OH-melagatran</th>
<th>Ethylmelagatran</th>
<th>Melagatran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TI</td>
<td>TI</td>
<td>TI</td>
<td>TI</td>
</tr>
<tr>
<td>AUC_{0-\infty} (h \cdot \mu M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>2.28 ± 0.66</td>
<td>1.88 ± 0.94</td>
<td>6.17 ± 1.15</td>
<td>0.54 ± 0.19</td>
</tr>
<tr>
<td>VH</td>
<td>1.17 ± 0.33</td>
<td>1.03 ± 0.51</td>
<td>4.38 ± 1.28</td>
<td>0.37 ± 0.16</td>
</tr>
<tr>
<td>VF</td>
<td>0.91 ± 0.26</td>
<td>0.85 ± 0.38</td>
<td>3.76 ± 0.72</td>
<td>0.37 ± 0.16</td>
</tr>
<tr>
<td>(C_{\text{max}}) (\mu M)</td>
<td>3.12 ± 1.43</td>
<td>2.11 ± 1.33</td>
<td>0.39 ± 1.90</td>
<td>0.33 ± 0.18</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>0.20 (0.2-0.5)</td>
<td>0.5 (0.2-0.8)</td>
<td>0.7 (0.5-1.5)</td>
<td>0.5 (0.5-1.5)</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>0.20 (0.2-0.5)</td>
<td>0.5 (0.2-1.2)</td>
<td>0.9 (0.5-1.9)</td>
<td>0.5 (0.5-1.5)</td>
</tr>
</tbody>
</table>

\* \(p < 0.05\).
on the apparent $E_H$ of ximelagatran, which was estimated as 43 ± 18 and 48 ± 9.2% after administration of ximelagatran with and without erythromycin, respectively. The apparent CL$_{H}$ of ximelagatran was 16 ± 3.2 ml/min/kg (ximelagatran administered alone) and 15 ± 6.0 ml/min/kg (ximelagatran and erythromycin administered), representing approximately 47% of its total CL. Excretion of ximelagatran, the intermediates, and melagatran into the perfused intestinal segment was negligible, implying that this route was of minor importance for the elimination of the compounds.

**Part II: Pharmacokinetics of Ximelagatran and Its Metabolites after i.v. Administration of Ximelagatran and Melagatran.**

The $t_{1/2}$ of melagatran varied according to whether the compound was administered i.v. as ximelagatran or as melagatran; it was twice as long after ximelagatran administration as after administration of melagatran ($p < 0.05$) (Table 5). The CL of ximelagatran and melagatran was 34 ± 2.1 and 6.4 ± 2.0 ml/min/kg, respectively, and the $V_{ss}$ was 0.31 ± 0.05 and 0.51 ± 0.08 l/kg, respectively.

The total amount eliminated in bile was 15 ± 7.9% of the i.v. melagatran dose and 17 ± 8.4% of the i.v. ximelagatran dose. Ethylmelagatran and melagatran were the predominating compounds in bile following i.v. administration of ximelagatran (Table 5). The route-dependent metabolic pattern in plasma, with higher plasma exposure of ethylmelagatran after i.v. ximelagatran administration than after enteral dosing, was also reflected in bile; after enteral administration the ranking of the amount excreted in bile was melagatran > ethylmelagatran, whereas the ranking after i.v. administration was ethyl-

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Ximelagatran</th>
<th>Melagatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP</td>
<td>F (%)</td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>38 ± 11</td>
<td>22 ± 3.5</td>
</tr>
<tr>
<td>TII</td>
<td>33 ± 18</td>
<td>28 ± 8.4*</td>
</tr>
<tr>
<td>VH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>19 ± 5.3</td>
<td>21 ± 3.7</td>
</tr>
<tr>
<td>TII</td>
<td>19 ± 8.8</td>
<td>28 ± 7.6*</td>
</tr>
<tr>
<td>VF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>12 ± 3.4</td>
<td>18 ± 3.2</td>
</tr>
<tr>
<td>TII</td>
<td>12 ± 5.4</td>
<td>24 ± 6.9*</td>
</tr>
</tbody>
</table>

*p < 0.05.
The direct thrombin inhibitor ximelagatran is known to be rapidly absorbed and bioconverted to its active form, melagatran, via two intermediate metabolites, OH-melagatran and melagatran. The apparent CL_bile of ethylmelagatran and melagatran was 8.8 ± 7.6 and 1.8 ± 1.2 ml/min/kg, respectively. The i.v. administration of melagatran resulted in a comparable melagatran apparent CL_bile of 1.2 ± 0.8 ml/min/kg. Ximelagatran, the intermediate metabolites, and melagatran were not detected in the perfusate sampled from the intestine throughout the experiment.

Discussion

The direct thrombin inhibitor ximelagatran is known to be rapidly absorbed and bioconverted to its active form, melagatran, via two intermediate metabolites, OH-melagatran and ethylmelagatran. Our major findings in the present study are that the bioconversion of ximelagatran in pigs is dependent on route of administration, that transport proteins mediate the hepatic uptake and/or biliary efflux of ximelagatran and its metabolites, and that these transporters are likely to be involved in the clinical interaction between ximelagatran and erythromycin.

The multiple sampling sites in this pig model and the different routes of administration provided further insight into the biotransformation of ximelagatran. Interestingly, the metabolism of ximelagatran was route-dependent, with different plasma concentration-time profiles for the intermediates after enteral and i.v. ximelagatran administration. OH-melagatran was the main intermediate in plasma following enteral ximelagatran administration but was barely detectable after i.v. administration. Thus, it is most likely that a high hydrolysis activity in the gut was involved in the first-pass metabolism of ximelagatran. The large estimated fraction of the ximelagatran dose absorbed suggests that most of this metabolism occurred after absorption across the apical membrane of the enterocytes. The difference in plasma exposure of the intermediates, depending on the route of administration, has not been observed in previous studies in humans and rats (Eriksson et al., 2003). The hydrolysis of the ethyl moiety on ximelagatran is catalyzed in vitro by unspecific CESs from pig liver (Clement and Lopian, 2003). In humans, two major CES isoforms have been identified: hCE-1 (a human CES1 isoenzyme) and hCE-2 (a human CES2 isoenzyme), with distinct tissue distribution and substrate specificity (Satoh et al., 2002; Imai et al., 2006). hCE-1 preferentially hydrolyzes ester-containing compounds that have a small alcohol and a large acyl group, and hCE-2 is more effective at hydrolyzing substrates with a large alcohol and small acyl moiety (Imai et al., 2006). This suggests that hCE-1 would be more effective than hCE-2 at catalyzing the hydrolysis of ximelagatran. In human liver microsomes, both hCE-1 and hCE-2 contribute to hydrolytic activity, whereas hydrolytic activity in human intestinal microsomes is caused by hCE-2 (Satoh et al., 2002). Hence, it can be hypothesized that following p.o. administration in humans, ximelagatran is not hydrolyzed until the compound reaches the liver, which is reflected by the similar plasma exposure of the intermediates after p.o. and i.v. ximelagatran administration (Eriksson et al., 2003). The formation of OH-melagatran and melagatran during passage through Caco-2 cell monolayers can be explained by the presence of hCE-1 in these cells (Imai et al., 2005; Eriksson et al., 2006). In pigs, the intestinal and liver CES isoforms are close to identical, exhibiting 77 and 76% sequence identity with hCE-1, respectively, and 47 and 46% sequence identity with hCE-2 (Musialowska-Persson and Bornscheuer, 2003). Assuming that similar substrate specificity follows with high sequence homology, the substrate specificities of pig intestinal and liver CESs would resemble that of hCE-1, resulting in hydrolysis of ximelagatran in the pig intestine and a route-dependent metabolism. These results show the importance of taking species differences in the expression and the catalytic efficacy of the CES isoforms into consideration when using animal data to predict metabolism and pharmacokinetics of ester-containing drugs in humans.

The enzyme system involved in the N-hydroxyamidine reduction seemed to be present in both hepatic and extrahepatic tissue in the pig, as observed from the reduction in plasma exposure of both ximelagatran and OH-melagatran from the VP to the VH and VF, that is, AUC_VP > AUC_VH > AUC_VF (Fig. 3). This is in accordance with data from in vitro studies showing that the enzyme system catalyzing the reduction of N-hydroxyamidine was active in microsomes and mitochondria from different organs, with the highest activity in renal and hepatic mitochondrial fractions (Clement and Lopian, 2003; Anderson et al., 2005). Apart from the route-dependent metabolism of ximelagatran, the pharmacokinetic profile of melagatran in pigs was in good agreement with results from clinical studies. For example, melagatran bioavailability after p.o. ximelagatran administration and
melagatran $V_{ss}$ are 19% and 0.3 l/kg, respectively, in humans (Johansson et al., 2003) and 19% and 0.5 l/kg, respectively, in pigs. In both humans (Eriksson et al., 2003) and pigs (Table 5), the $t_{1/2}$ of melagatran after i.v. administration of ximelagatran was approximately twice as long as that after i.v. administration of melagatran. This difference may be the result of the more lipophilic ximelagatran being able to gain access to cells that are harder for melagatran to enter, resulting in a greater $V_{ss}$ for melagatran after administration of ximelagatran (Johansson et al., 2003).

There were significant differences between the concentrations of the analytes in bile and plasma. In particular, for ethylmelagatran, the biliary concentration was 1000-fold higher than the plasma concentration following enteral administration of ximelagatran. The plasma concentrations were not representative of the concentrations determined in hepatic tissue either, a scenario that is important to keep in mind when evaluating, for example, possible drug-drug interactions and concerns for local tissue toxicity. The high concentrations of ethylmelagatran in hepatic tissue and bile imply, first, that N-reduction of ximelagatran was favored over ester hydrolysis in the hepatocytes, and, second, that ethylmelagatran is a possible substrate for efflux transporters at the canalicular membrane. Also, changing the route of administration of ximelagatran from enteral to i.v. resulted in the compound most excreted in bile changing from melagatran to ethylmelagatran. This can be explained either as greater hepatic uptake of ximelagatran after i.v. administration, followed by intracellular metabolism to ethylmelagatran and its efflux into bile, or increased plasma exposure of ethylmelagatran after i.v. ximelagatran administration reflected in the bile as a result of active uptake of ethylmelagatran at the sinusoidal membrane. Transport studies in Caco-2 cells and P-gp–transfected Madine-Darby canine kidney cells have indicated that ximelagatran is a substrate for P-gp (Eriksson et al., 2006). It has also been speculated that melagatran and OH-melagatran are transported by efflux transporters expressed at the apical membrane of the Caco-2 cells because these compounds were formed during the passage of ximelagatran through the Caco-2 monolayer and were preferentially excreted in the apical chamber (Eriksson et al., 2006). Our study also supports the involvement of transport proteins in the hepatobiliary transport of melagatran; the melagatran concentration in bile was 100-fold greater than that in plasma, and

TABLE 5

<table>
<thead>
<tr>
<th></th>
<th>Ximelagatran</th>
<th>OH-melagatran</th>
<th>Ethylmelagatran</th>
<th>Melagatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-6}$ (h·μM)</td>
<td>TIII 0.58 ± 0.07</td>
<td>0.03 ± 0.01</td>
<td>0.42 ± 0.34</td>
<td>0.73 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>3.71 ± 0.70</td>
</tr>
<tr>
<td>$AUC_{0-8}$ (h·μM)</td>
<td>TIII 0.58 ± 0.07</td>
<td>0.04 ± 0.01</td>
<td>0.47 ± 0.37</td>
<td>1.03 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>3.86 ± 0.80</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>TIII 0.2 ± 0.02</td>
<td>0.7 ± 0.3</td>
<td>2.0 ± 0.5</td>
<td>3.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>$f_{e, b}$ (% of dose)</td>
<td>TIII 0.05 ± 0.02</td>
<td>0.15 ± 0.20</td>
<td>10 ± 6.4</td>
<td>6.4 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>15 ± 7.9</td>
</tr>
<tr>
<td>$Cl_{bile}$ (ml/min/kg)</td>
<td>TIII 0.02 ± 0.01</td>
<td>0.50 ± 0.50</td>
<td>8.75 ± 7.61</td>
<td>1.83 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>1.15 ± 0.81</td>
</tr>
<tr>
<td>$Cl$ (ml/min/kg)</td>
<td>TIII 34 ± 2.1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>6.4 ± 2.0</td>
</tr>
<tr>
<td>$V_{ss}$ (l/kg)</td>
<td>TIII 0.31 ± 0.05</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>TIV N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.51 ± 0.08</td>
</tr>
</tbody>
</table>

N.A., not applicable.
the f sub b sub e was 0.15, which is high when considering the poor passive permeability of melagatran (0.03 ± 0.01 × 10⁻⁶ cm/s) (Gustafsson et al., 2001).

Erythromycin increased the plasma exposure of melagatran and reduced the biliary excretion of ximelagatran, intermediate metabolites, and melagatran in our study in pigs, results that are in line with previous studies in humans and rats (Erikkson et al., 2006). The pharmacokinetic interaction between ximelagatran and erythromycin is most likely not caused by direct inhibition of any of the metabolic processes affecting ximelagatran because the metabolism of this compound in plasma and bile and its bioavailability at all three plasma sampling sites were unaffected by erythromycin. Instead, inhibition of P-gp leading to reduced biliary excretion of melagatran has been suggested as a possible mechanism for the interaction (Erikkson et al., 2006). Erythromycin is a known inhibitor of P-gp and can therefore affect the biliary excretion of other P-gp substrates (Takano et al., 1998; Polli et al., 2001). More recently, it has also been suggested that erythromycin can modulate the hepatic disposition of concomitantly administered drugs by inhibiting uptake transporters such as the organic anion transporter 2 and various OATPs at the sinusoidal membrane (Fig. 5) (Kobayashi et al., 2005; Lam et al., 2006; Seithel et al., 2007). The ximelagatran-erythromycin interaction could also have occurred at transport protein sites in the intestine; however, there was no indication of any direct effect of erythromycin on the intestinal transport of ximelagatran and its metabolites. Ximelagatran was estimated to be well absorbed, and negligible amounts of the drug and its metabolites were excreted in the perfused intestinal segment during the experiment.

In conclusion, the bioconversion of ximelagatran in pigs was dependent on whether the compound was administered enterally or i.v. This observation has not been made in clinical studies presumably because of species differences in the expression and tissue distribution of CESs. Transport proteins expressed at hepatic sinusoidal/canalicular membranes play an important role in the hepatic disposition of ximelagatran and its metabolites; these transporters offer a likely site for the pharmacokinetic drug-drug interaction with erythromycin. Intestinal transporters appeared to be of minor importance for the disposition of ximelagatran and its metabolites.

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References


Address correspondence to: Hans Lennernäs, Professor in Biopharmaceutics, Department of Pharmacy, Uppsala University, Box 580, SE-751 23 Uppsala, Sweden. E-mail: hans.lennernas@farmaci.uu.se